

Cis – Trans Astaxanthin ratios

Trans astaxanthins are the predominant geometric isomers found in *Haematococcus pluvialis* algae (Yuan and Chan 1998), although studies have shown that the 9Z and 13Z cis isomers can be present in this species as much as up to 30% of the total trans/cis ratio (appendix 2, Aquasearch 2000i). In US Nutra's carbon dioxide extracted oleoresin, these ratios only change slightly (appendix 2), and due to the low level of total astaxanthin that will be used in the final encapsulated product, these differences appear to be of no significant importance. A literature survey has not revealed any toxicity issue with any of the astaxanthin skeleton based compounds. However, a recent single dose study of 100mg synthetically derived astaxanthin indicated that selective absorption does occur with preference to *cis* verses *trans* isomers in humans.

Geometric isomer	% of isomer prior to ingestion	% Human plasma levels after 7 hours from ingestion
All - <i>trans</i> E astaxanthin	74.0	49.4
9- <i>cis</i> astaxanthin	9.0	13.2
13 – <i>cis</i> astaxanthin	17.0	37.4

(Liaaen - Jensen et al., 2001)

This study indicates that the change in the 9-cis isomer increases by around 25% and the 13 – cis isomer by approximately 50% in human plasma. Such slight increases in these isomers through ingestion of 5mgs (total astaxanthin) of US Nutra's oleoresin compared to dried *Haematococcus* meal are therefore not expected to be of toxicological significance. Safety and toxicological studies highlighted in sections 5.2, 8.1 – 8.3 indicate that ingestion of much higher amounts of dried *Haematococcus pluvialis*, and therefore higher ingestion of cis astaxanthin isomers are unlikely to produce any toxic effects in humans. Moreover, astaxanthin fed Salmon have also been shown to accumulate both *cis* and *trans* astaxanthin, and no reported toxic effects on the large scale ingestion of Salmon with regard to these compounds has been reported in the scientific literature (Bjerkeng *et al* 2000).

References

- Bjerkeng B., Berge G.M., (2000). Apparent digestibility coefficients and accumulation of Astaxanthin E/Z isomers in Atlantic salmon (*Salmo salar* L.) and Atlantic halibut (*Hippoglossus hippoglossus* L.) Comparative Biochem. Physiol., Part B 127, 423 – 432.
- Liaaen - Jensen S., Bjerkeng B., Osterlie M., (2001). Cis-Trans Isomerism of Carotenoids – in relation to Nutrition and Health, Malaysian Oil Science and Technology, Vol 1, No.1 p1-10.
- Yuen, J.P. and Chen ,F. (1998). Chromatographic separation and purification of *trans* – isomers from the extracts of *Haematococcus pluvialis*. J. Agric. Food Chem. 46, 1952-1956.